

Summary of significant accomplishments of the PL.480
Project 01-062-1 "Physiological Role of Bacterial Ribonucleases"

- i) RNase I of *Salmonella typhimurium* has been purified to homogeneous state (M.W.20,000) as judged by polyacrylamide gel electrophoresis. Its properties have been studied. RNase II has also been extensively purified. RNase III has been obtained practically in homogeneous state (M.W.44,000).
- ii) A single step procedure of separation of RNases I, II and III present in the extract of *S. typhimurium* has been developed. The different affinities of RNases with the ribosomes and DEAE-cellulose have been utilized for the purpose.
- iii) A method of preparation of ribosomes without the help of ultracentrifuge has been developed. This involves ammonium sulphate fractionation, gel filtration through G-100 column and DEAE-cellulose chromatography.
- iv) A RNase I minus mutant (MB24) of *S. typhimurium* has been isolated by treatment of the wild strain LT2 with nitrosoguanidine. The mutant has been of great help in furtherance of the work under the project.
- v) Mechanism of association of RNases, especially of RNase I with the ribosomes and their subunits, has been extensively studied. RNase I specifically associates with the 30s subunit of 70s ribosomes and one 70s subunit binds with approximately 3 molecules of enzyme. Mg^{++} -dependent inhibition of RNase I catalysed hydrolysis of polyribonucleotides by 70s ribosomes and 30s subunit has been utilized for binding studies. Gradual removal of proteins from 70s ribosome by salt treatment leads to gradual loss of its inhibitory capacity. Inhibitory capacity is restored by the reconstitution of the ribosomes from the salt-treated particles and the proteins detached. On the basis of these results a simple assay method has been developed for following the extraction of proteins from the ribosomes and the reconstitution of the ribosomes from the salt-treated particles and the detached proteins.
- vi) Unlike 70s ribosomes and 30s subunits, the larger subunits (50s) are attacked by RNase I even in presence of high concentration of Mg^{++} . However, 70s ribosomes as well as 30s subunits are also amenable to the attack of RNase I provided the enzyme is added in excess of what is made-latent by 30s subunits. Unlike *S. typhimurium* and *E. coli* ribosomes and their subunits, *L. plantarum* ribosomes and their subunits are readily attacked by the enzyme in presence of even limiting amount of RNase I and high concentration of Mg^{++} . The importance of ribosomal configuration is also emphasized by the observation that streptomycin protects *E. coli* 70s ribosomes and 30s subunits (but not larger subunits) against the attack of RNase I at low Mg^{++} concentration and the number of enzyme molecules bound to 30s subunit is increased three fold in presence of streptomycin.